Clinical Review – Restylane (P020023)

Introduction

The basis of pre-marketing application P020023 is the outcome of a prospective Pivotal Clinical Study performed in the US under investigational device exemption along with an open label extension.

P020023 also contains an uncontrolled copy of the report of a non-randomized an unmasked study of 112 patients that was conducted in Sweden during 1995-6 with an earlier formulation of Restylane. The cohort in this non-randomized and uncontrolled study included non-pregnant and non-lactating patients with depressed cutaneous scars and one to three facial wrinkles up to 4mm in depth; patients were excluded if other local wrinkle therapy had been administered within 6 months prior to study due to potential confounding of Restylane's safety and effectiveness. Of this cohort, 20 patients were randomly selected for follow-up at 52 weeks. As the device has been re-formulated, data from this non-randomized, uncontrolled, and unmasked study is included only in discussion of device safety.

Pivotal Study

?? ***Devices***

Investigational: Restylane is a stabilized, bacterium – generated (non-animal) hylauronic acid suspended in physiologic buffer at pH = 7 and concentration of 20mg/ml, intended for use to correct contour deformities, e.g., naso-labial wrinkles. Delivered during study via 0.7 cc syringe and 30 gauge x 1/2"" needle. Maximum dose / treatment session: 1.5 ml.

Control: Zyplast cross-linked collagen Implant is purified bovine dermal collagen cross linked with glutaraldehyde, dispersed in phosphate buffered saline and 0.3% lidocaine, indicated for the correction of contour deficiencies of soft tissue. Delivered during study via 1.0 cc syringe and fine gauge needle. Maximum dose Zyplast / year: 30 ml.
Zyderm, the non-crosslinked collagen implant analog, was used for pre-treatment skin testing for hypersensitivity to bovine collagen.

?? ***Design***

Highlights

The pivotal study was a 1 to 1 randomized, prospective study conducted at 6 US centers to compare Restylane and Zyplast in a within patient control model of augmentation correction of bilateral nasal labial folds: the randomized side was treated with Restylane; the opposite side was treated with Control. Treatment was considered to be complete when optimal correction as determined by treating physician discretion, not by a pre-determined change in objective measure, was found to be sustained for 2 weeks after injection. This follow-up 2 weeks post-initial or touch-up injection began the 'Baseline' for 6, 9 and 12 month follow-up. Effectiveness was studied with 6 month follow-up from 'baseline'. Safety was studied from initial treatment and touch-up as needed to achieve optimal correction that was sustained for 2 weeks, through 12 month post- 'baseline' follow-up.

Masking Plan

?? Patient: partially masked
?? Evaluating physician: independent and masked
?? Treating physician: unmasked

Primary Objectives

The pivotal study primary objective was to evaluate the safety and effectiveness of Restylane compared to Control in patients seeking augmentation correction of bilateral nasal labial folds that met study criteria.

?? Effectiveness: the primary objective was to evaluate differences in effect of Restylane and Control on the visual severity of the nasolabial folds, as assessed by an Evaluating Investigator at 6 months post-'baseline'.

Optimal correction was defined to be the best cosmetic result obtainable with 2 injectable implants as determined by the evaluating physician; a specific objective score or goal for optimal correction was not defined. The evaluation parameter was the Wrinkle Severity Rating Scale (SRS) Score:

1. Absent: no visible fold; continuous line
2. Mild: shallow but visible fold with slight indentation; minor facial feature.
3. Moderate: moderately deep fold; clear facial feature visible at normal appearance but not when stretched. Excellent correction expected.
4. Severe: very long and deep; prominent facial feature; less than 2mm visible fold when stretched.
5. Extreme: extremely deep and long folds; 2-4mm visible v-shaped fold when stretched; detrimental to appearance; unlikely to have satisfactory correction with injectable implant alone.

This scoring system was validated per review of 30 non-study photos by Evaluating Investigators. Based on this photo review, an SRS change = 1 was considered to be clinically significant. Validation was not confirmed by evaluation of pivotal study photos.

?? Safety: the pivotal study primary objective was evaluation of adverse events recorded by
   ?? Patient Diary: intensity and duration of pain, tenderness, swelling, redness, bruising and itching for 14 days post-treatment.
   ?? Follow-up by the unmasked treating investigator from treatment through 12 months.

Hypersensitivity reaction was considered as to Restylane and Control. Pre-screening skin testing for sensitivity to the cross-linked collagen Control, Zyplast, was performed using the non-crosslinking analog, Zyderm. Pre-screening skin test for sensitivity to the bacterial source hyaluronate, Restylane was not performed due to low suspicion of hypersensitivity. No anti-body titers were drawn pre-treatment to collagen or to hyaluronate. Post-treatment adverse event skin testing was planned to evaluate sensitivity to hyaluronate and collagen in case hypersensitivity reaction was suspected by the unmasked treating investigator during follow-up. Criteria with protocol details are listed in Appendix 1.

Secondary objectives

?? SRS score assessed at 2, 4, and 6 months post-'baseline' by the evaluating investigator and by the subject.
?? Number of treatment sessions needed to achieve optimal cosmesis.
?? Global Aesthetic Improvement (GAI): a subjective, non-validated scale assessed at 2, 4, and 6 months by the evaluating investigator and by the subject:
    ?? Very much improved
    ?? Much improved /
    ?? Improved
    ?? No change
    ?? Worse

Study Population Criteria

Highlights:

?? Non-pregnant, non-lactating adults seeking augmentation correction of bilateral nasolabial folds.
?? SRS 3 or 4 at pre-treatment evaluation
?? Willing to abstain during the study from exclusion procedures, e.g.: Laser or chemical re-surfacing, Botox injections, aesthetic facial surgery, concurrent facial wrinkle treatments, immuno-modulary therapy, desensitization injections to meat products.
?? Without active skin disease within 6 months of study entry, known connective tissue disease or immunosuppressive therapy.
?? Without any aesthetic facial therapy within 6 months of study entry.
?? Without coagulopathy or known allergy / hypersensitivity or planned desensitization to device components or meat products.

Inclusion and Exclusion Criteria with protocol details are in Appendix 2.

Study Procedure

The pivotal study procedure consisted of 2 phases:

During the Treatment Phase, device doses were provided as required to achieve optimal cosmetic result, within maximum limits per device. Patients were re-evaluated every two weeks with touch-up if correction was sub-optimal on follow-up. The 'baseline,' i.e.: post-treatment baseline, began at the visit at which optimal correction had been maintained for 2 weeks since last treatment.

Follow-up occurred by two schedules:

?? Effectiveness: At 2, 8, 16 and 24 weeks after 'baseline'
?? Safety: At 2, 8, 16, 24, 36 and 52 weeks after 'baseline'

Sample Size

Sample size determination was based on the hypothesis that three times as many Restylane treated sites would remain superior compared to control at 6 months after 'baseline'. Superiority per patient was defined as a difference of at least 1 in the SRS score in favor of one of the treatments. At any time, SRS per patient is determined in whole units of SRS as the Wrinkle SRS is an integer scale. An SRS score

difference or change = 1 was considered to be clinically significant based on the non-study photo validation study.
Minimum enrollment, accounting for potential loss to follow-up, was statistically determined to be N = 130 patients.

?? **Pivotal Study Outcomes**

Demographics

On the basis of this design, the study enrolled a population of predominately healthy, female, Caucasian non-smokers with minimal sun exposure. There were few men or other racial / ethnic groups; few smokers or patients with extensive sun exposure. Reference: Table 11.2, P020023, p895.

?? Gender

Male:	9 (6.6%)
Female:	128 (93.4%)

?? Ethnicity

Caucasian:	122 (89.0%)
Black:	2 (1.5%)
Asian:	2 (1.5%)
Hispanic:	11 (8.0%)

?? Tobacco use

Non-smoking:	118 (86.1%)
Smokers:	19 (13.9%)

?? Sun Exposure

None:	83 (60.6%)
Natural Sun:	52 (38.0%)
Artificial:	2 (1.5%)

A total of 48 patients (35.0%) had not had any previous facial aesthetic procedures; data was missing for 6 patients; 83 patients (60.6%) had had prior facial aesthetic procedures.
Reference: Table 11.4 & 5, P020023, p897.

?? Collagen injection	59 (43.1%)
?? Botulinum toxin injection	32 (23.4%)
?? Face-lift	16 (11.7%)
?? Laser Resurfacing	15 (11%)
?? Chemical resurfacing	12 (8.8%)
?? Autologous fat transplant	5 (3.6%)
?? Other	23 (16.8%)

Patient Disposition

Number of Subjects presenting at each follow-up time point:

?? Pre-treatment	138

?? ‘Baseline’* 138
?? 6 months 134**
?? 9 months 125 for safety***
?? 12 months 125 for safety

*‘Baseline’ defined as the 2 week follow-up point at which optimal correction has been maintained for 2 weeks.
** 4 Patients were withdrawn / lost to follow-up before 6 months.
*** 9 Patients were withdrawn / lost to follow-up before 9 months

Evaluating Investigator & Patient Masking Assessment

Evaluating investigator & patient masking assessment found that the incidence of correct guess as to treatment, for both the evaluating investigator and patients, increased during the study from approximately 60% correct guess at baseline to 70% correct guess at 6 month follow-up. Masking was found to vary significantly by center. An incidence of correct guess greater than 50% is considered to suggest incomplete masking. Therefore study masking was incomplete from baseline and progressively less effective during the trial. Reference: Table 12.12 – 13, P020023, p915-18.

		Evaluating Investigator	Patient
Baseline	Correct	***88 (64.2%)***	***82 (59.8%)***
	Not correct	47 (34.3%)	46 (33.6%)
	Total reporting	135 (98.5%)	128 (93.4%)
Month 2	Correct	***91 (66.4%)***	***82 (59.8%)***
	Not correct	38 (27.7%)	41 (29.9%)
	Total reporting	129 (94.2%)	123 (89.8%)
Month 6	Correct	***96 (70.1%)***	***93 (67.9%)***
	Not correct	37 (27.0%)	38 (27.7%)
	Total reporting	133 (97.1%)	131 (95.6%)

Primary Effectiveness

Comparative SRS per patient at 6 months as determined
By the evaluating investigator:
N = 137
Restylane lower (better) than Control: 80
Restylane equal to Control: 44
Restylane higher (worse) than Control: 13
Reference: P020023 A5, tab11, response to Question 10 (Appendix 3 of this review)

With both treatments, Restylane and Control, a mean 1.5 unit improvement of SRS was made from pre-treatment to establish optimal correction: post-treatment ‘baseline’ or month 0.

SRS: Pre-treatment to Optimal:	0month	2month	4month	6month
N =	137	137	137	137
Restylane	0	1.5	1.25	1.01	0.93
Control	0	1.52	0.94	0.54	0.36
Difference	0	0.02	0.31	0.47	0.57

Reference: P020023 A3, appendix 25

Mean SRS Score
By evaluating investigator:

	N	Restylane	Control	Absolute Difference*
Pre-treatment	138	3.29	3.31	0.02
Baseline	137	1.80	1.79	0.01
6 months	134	2.36	2.94	0.58

*between Restylane and Control
Reference: P020023 A5, tab11, response to Question 10 (Appendix 3 of this review)

Data demonstrates that while there was essentially no difference between Restylane and Control treated cohort sides at pre-treatment (0.02 Units SRS) and baseline (0.01 Units SRS), for the cohort of 134 patients, there was a difference of 0.58 units of SRS at 6 months.

The difference in treatment effect for the cohort of 134 to 138 patients based on SRS plateaus at about 0.58 at 6 months post 'baseline'. A difference in SRS of 1 is considered to be clinically significant per pre-study validation of the SRS scale.

Secondary Objectives

?? Comparative SRS per patient at 6 months as determined
By Patients
N = 137
Restylane greater (worse) than Control: 8
Restylane lower (better) than Control: 76
Restylane equal to Control: 53
Reference: P020023 A5, tab11, response to Question 10 (Appendix 3 of this review)

?? Mean SRS Score
By Patients

	N	Restylane	Control	Absolute Difference
Pre-treatment	138	3.33	3.37	0.04
Baseline	138	1.96	1.97	0.01
6 months	134	2.44	3.01	0.57

Reference: P020023 A5, tab11, response to Question 10 (Appendix 3 of this review)

?? Global Aesthetic Improvement

By Evaluating Investigator

Follow-up:	0month	2month	4month	6month
N	134	136	137	137
%Restylane > Control	3.6	38.7	56.9	62
%Restylane = Control	89	52.6	34.3	29.9
%Restylane < Control	5.1	8	8.8	8

Reference: P020023, Table 12.9, p913

By Patient Evaluation

Follow-up:	0month	2month	4month	6month
N	133	136	137	137
%Restylane > Control	11.7	34.3	43.1	55.5
%Restylane = Control	75.9	55.5	47.4	36.5
%Restylane < Control	9.5	9.5	9.5	8

Reference: P020023, Table 12.11, p914

With time post-optimal cosmesis, comparing Restylane and Control, report of the global aesthetic improvement score favoring Restylane increased. This trend was similar for data by evaluating investigators and patients.

?? Number of treatment sessions to achieve optimal cosmesis was evaluated.

For both Restylane and Control, optimal cosmesis required 1 to 3 treatments.

Optimal Cosmesis with initial treatment alone:

?? Restylane: n = 89 (65.0%)

?? Control: n = 85 (62.0%)

Optimal Cosmesis requiring 3 treatments:

- ?? Restylane: n = 7 (5.1%)
- ?? Control: n = 3 (2.2%)

Overall, no statistically significant different numbers of treatments were required to achieve Optimal Cosmesis with Restylane or Control.

Safety

?? *Restylane:* Basic criteria used for some of the more frequent types of reaction observed after treatment with Restylane were as follows. Reference: P020023, p965; P020023 A3, p6.

Hypersensitivity: inflammatory reaction with swelling, redness, tenderness, induration and rarely acneform papules at the injection site with an onset of one to several weeks after the initial treatment in

individuals not previously treated, and in < 7 days following treatment in patients known to have been previously exposed. Average duration 2 weeks.

Injection site reaction: a mix of different types of reactions that do not fit with other classifications: mainly short-term inflammatory symptoms starting early after treatment and with < 7 days duration.

?? *Control*: Basic criteria used for some of the more frequent treatment responses reported in Labeling for Control were as follows. Reference: P020023, p999-1000.

Hypersensitivity: reactions have occurred in 1 – 2% of treated patients: erythema, swelling, induration, and / or urticaria at implant sites. Typically reactions persist between 1 and 9 months; average duration of 4 months.

Rarely, reactions resolve in 1 or 2 weeks, or last more than 1 year. Rarely, abscess formation occurs, in some cases associated with elevated anti-bovine collagen antibodies, weeks to months following injections and may cause induration and / or scarring. Most have occurred in patients who became sensitized to collagen implants at some point during treatment.

Injection site reaction: minimal swelling, redness, and discomfort will probably occur immediately following implantation. Temporary palpable lumpiness or visible material (white papules or milia-like yellow) may occur.

?? Maximal intensity: After the initial session
Reference: P020023, Table 13.9, p 930

	Restylane side	Zyplast side	Restylane side				Zyplast side			
	Total reporting symptoms n (%)	**Total reporting symptoms n %~**	**None n (%)**	**Mild n %~**	**Mode-rate n (°/~~**	**Severe** n (%)	**None** n (%)	**Mild** n (%)	**Mode-rate** n %~	**Severe** n (%)
Bruising	72 (52.2) -	67 (48.6)	63 (45.6)	32 (23.2)	35 (25.4)	5 (3.6)	68 (493)	43 (31.2)	23 (16.7)	1 (0.7
Redness	% l l7 (84.8)1	117 (84.8)	17 (12.3)	56 (40.6)	54 (39.1)	7 (5.1)	17 (12.3)	72 (52.2)	37 (26.8)	8 (5.8
Swelling	120 (87.0)	102 (73.9)	14 (10.1)	54 (39.1)	61 (44.2)	5 (3.6)	32 (23.2)	65 (47.1)	35 (25.4)	2 (1.4
Pain	79 (57.2)	58 (42.0)	55 (39.9)	40 (29.0)	34 (24.6)	5 (3.6)	76 (55.1)	46 (33.3)	10 (7.2)	2 (1.4
Tendernes	107 (77.5)	89 (64.5)	27 (19.6)	60 (43.5)	43 (31.2)	4 (2.9)	45 (32.6)	70 (50.7)	17 (12.3)	2 (1.4
Itching	42 (30.4)	33 (23.9)	91 (65.9)	31 (22.5)	11 (8.0)	0 (0.0)	101 (73.2)	27 (19.6)	6 (4.4)	0 (0.0
Other	34 (24.6)	33 (23.9)	93 (67.4)	14 (10.1)	15 (10.9)	5 (3.6)	94 (68.1)	20 (14.5)	10 (7.2)	3 (2.2

Data indicate that there was an increased incidence of bruising, swelling, pain, tenderness and itching after first treatment with Restylane compared to Control.

?? Maximal intensity: After touch-ups
Reference: P020023, Table 13.10, p 931

	Res lane side		Z plast side		Restylane side				Zyplast side ,			
	Total reporting n	Not reporting n	Total reporting n	Not reporting n	None n	Mild n	Mode-rate n	Severe n	None n	Mild n	Mode-rate n	Severe n
Bruisin	43	6	44	9	24	11	6	2	25	11	6	2
Redness	43	6	44	9	8	16	14	5	5	18	17	4
Swelling	43	6	44	9	8	18	14	3	9	23	10	2
Pain	43	6	44	9	21	14	7	1	29	7	7	1
Tendernes	43	6	44	9	9	19	13	2	16	19	8	1
Itching	43	6	44	9	34	5	2	2	31	6	5	2
Other	41	8	44	9	36	3	2	0	38	0	6	0

The incidence of most reactions was lower for both treatments after the touch-up injections, than after the initial injection.

P-values for difference between treatments after initial session and after all touch-ups comparing Restylane and Control categories: none/mild and moderate/severe.
Reference: P020023 A3, Tab 11, response to Question 3b.

	After initial session	After all touch-ups
N	Control, n = 138	Control, n = 53
	Restylane, n = 138	Restylane, n = 49
Bruising	0.0025	1.000
Redness	0.0139	1.000
Swelling	<0.0001	0.125
Pain	<0.0001	1.000
Tenderness	<0.0001	0.125
Itching	0.0625	0.2500
Other	0.1185	0.2500

This table presents p-values for difference between Restylane and Control reports of 2 groups: those with none or mild symptoms, and those with moderate or severe symptoms, and demonstrates that there was a statistically significant difference between treatments as to maximal symptom intensity for bruising, redness, swelling, pain and tenderness, as well as a trend towards a statistically significant difference for itching.

Symptom Duration: after initial session
Reference: P020023, Table 13.11, p 932

!~	Restylane side		Zyplast side		Restylane side				Zyplast side			
	Total repor -tin-, n	Not repor -frog n	Total repor -tmg n	Not repor -tine n	1 n (%)	Number 2-7 n (%)	of 8-13 n (%)	14- n (%)	1 n (%)	Numb 2-7 n (%)	of days 8-13 n	14-~
Bruising	135	3	135	3	7 (5.1)	56 (40.6)	6 (4.4)	3 (2.2)	7 (5.1)	53 (38.4)	5 (3.6)	2 (1.4)
Redness	134	4	134	4	19 (13.8)	68 (49.3)	18 (13.0)	12 (8.7)	19 (13.8)	71 (51.4)	15 (10.9)	12 (8.7)
Swelling	134	4	134	4	16 (11.6)	84 60.9)	16 (11.6)	4 (2.9)	14 (10.1)	70 (50.7)	16 (11.6)	2 (1.4)
Pain	134	4	134	4	29 (21.0)	48 (34.8)	2 (1.4)	0 31 (0.0) (22.5)		25 (18.1)	1 (0.7)	1 (0.7)
Tenderness	134	4	134	4	21 (15.2)	78 (56.5)	6 (4.4)	2 27 (1.4) (19.6)		54 (39.1)	6 (4.4)	2 (1.4)
Itching	133	5	134	4	' 11 (8.0)	25 (18.1)	6 (4.4)	0 8 (0.0) (5.8)		22 (15.9)	3 (2.2)	0 (0.0)
Other	127	11	127 11		7 (5.1)	23 (16.7)	3 (2.2)	1 10 (0.7) (7.2)		15 (10.9)	6 (4.4)	2 (1.4)

?? Symptom Duration: after all touch-ups
Reference: P020023, Table 13.12, p 932

	Restylane side	Zyplast_sidee	Restylane _side _				Zyplast side			
	Total reporting symptoms n	Total reporting symptoms n	Number of days 1 n	2-7 n	8-13 n	14- n	Number of da s 1 n	2-7 n	8-13 n	14 n
Bruising	19	19	3	12	3	1	2	14	1	2
Redness	35	39	4	18	6	7	6	15	6	12
Swellimv	35	35	4	24	4	3	8	20	4	3
Pain	22	15	11	10	! 1	0	6	9	0	0
Tendernes	34	28	6	26	2	0	7	16	5	0
Itching	9	13	3	3	3	0	2	6	3	2
Other	5	6	1	3	1	0	0	2	2	2

Clinical trials have not evaluated anti-body titers before or after treatment with Restylane to allow correlation of symptoms with immune response, and so to objectively characterize the symptom profile associated with immune response to Restylane. The overlap of symptom profiles for Restylane hypersensitivity and injection site reactions, and lack of correlation of symptoms with anti-body titers, may have confounded diagnosis of hypersensitivity reaction to the investigational device during the pivotal trial.

Open Label Extension

?? ***Design***

The open label extension of the pivotal trial allowed study participants to receive uni-lateral or bilateral re-treatment with Restylane at the 6 or 9 month visits, while continuing accrual of safety data for the pivotal trial.

?? For patients who were re-treated, efficacy was assessed before re-treatment, not after re-treatment.

?? For patients who were not re-treated, no efficacy assessment was made beyond 6 months.

Hypotheses:
Efficacy: Restylane is superior to Zyplast 3 times as frequently as Zyplast is superior to Restylane.
Safety: pivotal protocol continued.

?? ***Outcomes***

Number of Patients:	for Safety	for Effectiveness	Re-treated
Pre-treatment	138	138	
Baseline	138	138	
6 months	134*	134	100
9 months	125 for safety**	34	28
12 months	125 for safety	7	

Assessment of Pivotal Study treatment effectiveness at 9 or 12 months is limited as
100 of 138: 72.5 % of Pivotal Study patients were re-treated at 6 months.
34 of 138: 24.6 % of Pivotal Study patients presented for effectiveness at 9 months.
7 of 138: 5.0 % of Pivotal Study patients presented for effectives at 12 months.

* 4 patients withdrawn / lost to follow-up before 6 months
** 9 patients withdrawn / lost to follow-up before 9 months

Overall Summary

Effectiveness

?? Optimal correction is achievable with both Restylane and Control by a mean 1.5 unit SRS increase, in a comparable number of sessions.

?? Wrinkle SRS assessment that 1 unit is a clinically significant change was not confirmed on study photos.

?? SRS at 6 months was clinically significantly (1 unit, minimum) higher for Restylane than Control in 59.7% patients, but less than clinically significantly higher (0.6 unit) for the overall cohort.

?? SRS at 9 and 12 months post-treatment is limited as most (72.5%) patients were re-treated at 6 months.

Safety

?? Hypersensitivity reaction to Restylane is reported to vary in onset and symptom presentation, possibly representing different mechanisms of reaction.

- Symptoms of inflammation within 14 days post-treatment were of statistically significantly higher intensity after initial treatment with Restylane compared to Control.
- Two papule / nodule lesions reported with onset at more than 40 days post treatment.

?? Injection reaction and early hypersensitivity symptom profile overlap and may confound diagnosis of hypersensitivity reaction to a new product: hypersensitivity reaction may have been unrecognized.
?? Anti-body titer not evaluated; symptom profiles have not been correlated to immunologic change.

Appendix 1
Reference: P020023, p965 - 966
Hypersensitivity Reactions

Reactions thought to be of a hypersensitivity nature have been reported in about one in every 2,000 subjects treated with Restylane®, and up to 3% of subjects treated with Zyplast® (who had a negative skin test).

In the case of Restylane® these reactions consist of swelling and induration at the implant site, sometimes with edema in the surrounding tissues. Erythema, tenderness and rarely acneiform papules may also occur. The reactions started either shortly after injection or after a delay of 2-4 weeks and were described as mild to moderate., self-limiting with an average duration of 2 weeks. In pronounced cases a short course of oral steroids may prove effective.

Similar reactions have occurred in subjects receiving ZyplastO as well as more severe systemic reactions.

If an adverse reaction occurred indicating a hypersensitivity etiology, the subject was to be followed-up according to the schedule below:
1. When the subject had been free of symptoms for at least two weeks, a volume of 0.1 mL of Restylane® was implanted intradermally on the volar aspect of the left forearm, and a Collagen Test Implant on the volar aspect of the right forearm. The subject was to be instructed to visually inspect the test site for reactions (see below) and to be especially observant during the following three days.

Note: the subject was also to be instructed not to aggravate: the test site by scratching or repeatedly touching it. On the third day following the test injection, the Investigator was to inspect the injection site.

2. A positive test response was defined as: any change in the original welt (such as increased erythema, induration, tenderness or swelling) with or without accompanying pruritus, which persisted for more than six hours and appeared more than 2.4 hours after implantation.

3. The readings were scored as follows:
?? No reaction
?? Doubtful reaction
?? Weak reaction (erythematous and maybe papular)
?? Strong reaction (erythematous and edematous or vesicular)

If the test response was judged to be doubtful, a second test dose was to be administered at this same visit on the opposite arm and evaluated/scored as described above.

4. In case of a positive test response (weak or strong reaction) to the Restylane® test dose, a skin sample was obtained from the test site with a punch biopsy (2 mm). The tissue specimen was then placed in a standard buffered formaldehyde fixative and sent to a pathologist (local laboratory) for immuno-histochemical examination with the differential diagnosis "cell-mediated hypersensitivity reaction."

Appendix 2

Reference: P020023, p857 - 9

All inclusion and none of the exclusion criteria were to be met at the treatment visit, before the treatment was given to the subject.

9.4.1 Inclusion Criteria

1. subjects who were males or non-pregnant, non-breast-feeding females' aged 18 years or older; and
2. were outpatients seeking augmentation therapy for correction of bilateral nasolabial folds. The subjects should have a score of 3 or 4 on the Severity Rating Scale; and
3. had the ability to understand and comply with the requirements of the study; and
4. were willing to abstain from exclusionary procedures (e.g., further augmentation therapy.. laser or chemical resurfacing; Botox(k injections; facelift) for the duration of the study; and
5. gave written informed consent to participate in the study.

9.4.2 Exclusion Criteria

Subjects with any of the following criteria were to be excluded from the study:

1. subjects with active skin disease, inflammation or related conditions, such as infection, psoriasis and herpes zoster near or on the nasolabial folds
2. subjects that had undergone procedures based on active dermal response (e.g. laser and chemical peeling procedures) below the level of the lower orbital rim, within 6 months prior to randomization
3. use of any facial tissue augmenting therapy or aesthetic facial surgical therapy effecting areas below the level of the lower orbital rim, within six (6) months prior to randomization, e.g. injection or other form of implantation of tissue augmenting substances, Botox® injections or facelift
4. use of facial wrinkle therapies, including Accutane® or Renova® within six (6) months prior to study entry
5. concomitant anticoagulant therapy, antiplatelet therapy, or a history of bleeding disorders
6. a history of allergies to any bovine collagen products or a positive response to the Collagen Test Implant (administered at screening)
7. a history of severe allergies manifested by a history of anaphylaxis, or a history or presence of
8. multiple severe allergies
9. known lidocaine hypersensitivity
10. known latex allergy
11. subjects undergoing or planning to undergo desensitization injections to meat products, as these
12. injections can contain bovine collagen
13. a presence or history of connective tissue diseases (e.g., rheumatoid arthritis, juvenile rheumatoid arthritis, scleroderma, or systemic lupus erythematosus)
14. subjects on immunosuppressive therapy

' Women of childbearing potential had to use a medically acceptable method of birth control, and had to have a negative urine pregnancy test at Visit 2, prior to treatment.

Appendix 3
Efficacy results up to 12 months
Question 10

ITT population at 6 months, n=137 ITT population at 9 months, n=34 ITT population at 12 months, n=7

		SRS -evaluator_ Restylane	Control	-	SRS -subject -_ Restylane	Control	
Pre-treat n		137	137		137	137	
	Mean	3.29	3.31		3.33	3.37	
	Median	3	3		3	3	
	Range	2-4	2-4		2-5	2-5	
	SD	0.54	0.54		0.54	0.56	
Baseline n		137	137		137	137	
	Mean	1.80	1.79	0.84	1.96	1.97	0.70
	Median	2	2		2	2	
	Range	1-4	1-4		1-5	1-4	
	j SD	0.68	0.66		0.81	0.79	
6 months 11		137	137		137	137	
	Mean	2.36	2.94	<.0001	2.44	3.01	<.0001
	Median	2	3		2	3	
	Range	1-4	1-4		1-5	1-5	
	SD	0.78	0.76		0.80	0.75	
Incidence of							
SRS Restylane > Control		13		<.0001	8		<.0001
SRS Restylane < Control		80			7		
SRS Restylane = Control		44			5		
9 months n		34	34		34	34	
	Mean	2.44	3.12	<.0001	2.47	3.12	<.0001
	Median	2.5	3		2	3	
	Range	1-4	2-4		2-5	2-5	
	SD	0.86	0.59		0.75	0.64	
Incidence of							
SRS Restylane > Control		0		<.0001	0		<.0001
SRS Restylane < Control		21			2		
SRS Restylane = Control		13			1		
12 months n		1 3	3		3	3	
	miss	4	4		4	4	
	Mean	2.67	3.00	N.A.	2.00	2.67	N.A.
	Median	3	3		2	3	
	Range	2-3	3-3		2-2	2-3	
	SD	0.58	0		0	0.58	
Incidence of							
SRS Restylane > Control		0		N.A.	0		N.A.
SRS Restylane < Control		1			2		
SRS Restylane = Control		2			1		

p-value for the mean: Student's paired t-test p-value for the incidence:
McNemar's test
No tests has been performed for 12 months data, since only 3 subjects had values

Missing data at baseline, 2,4,6 and 9 months is substituted with tl,e pre-treatment value

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ITT population at 6 'months, n=13 ;'

ITT population at 9 months, n=34

ITT population at 12 months, r_=7

		SRS-evaluator			SRS-subject		
		Restylane -	Control	; p	Restylane	Control_	p
Change from Pre-treatment to baseline	n	137;	137		137	137	
	Mean	1.50	1.52	0.55	1.37	1.40	0 48
	Median	1	1		1 ...	1	
	Range	0-3	0-2		-1 - 3	-1 - 3	
	SD	0.67	0.64		0.82	0.75	
Change from pre – treatment to 2 months	n	137	13 7'		137	137	
	Mean	1.25	0.94	<001	1.08	0.89	0.0019
	Median	1	l		1	1	
	Range	0 – 3	0 - 2		-2 - 4	-2 - 2	
	SD	0 67	0 68		092	0 82	
Change from pre-treatment to 4 months	n	137	137		137	137	
	Mean j	1.01	0.54	<.0001	0.83	0.56	<.0001
	Median	1	0		1	0	
	Range	-1 - 2	-1 - 2		-1 - 3	-1 - 3	
	SD	OAS	0.6-'		0.90	0.-6	
Change from pre-treatment to 6 months o	n	137	137		137	137	
	Mean	0.93	0.36	<.0001	0.89	0.36	<.OO0l
	Median	1	0		1	0	
	Range	0-3	-10		03	-~_	
	SD	0.75	0.68		0.87	0.77	
Change from pre-treatment to 9 months	n	34	34		34	34	
	Mean	0 65	-0.03	<0.OOl	0.71	0.09	< 0001:
	Median		0		i	O	
	Range ^	0-2	-1-0		-1-2	-2-1	
	SD	0.54	0.17		0.68	0.54	
Change from pre-treatment \ to 12 months	n	3	3		3	3	
	Miss	4	-*		4	4	
	Miss	0.33	0	n.a..	1.00	0.33	na.
	Median	0	0	i	1	0	
	Range	0-1	0		1	0-1	
	SD	0.58	0		0	0.58	

p-value for the mean: Student's paired t-test

No tests has been performed for 1'2 months data, since only 3 subjects had values

Missing data at baseline. 2.4, 6 and 9 months is substituted with the pre-treatment value